

Attorney Docket No.: P-756 (TI-0022)
Inventors: Huber et al.
Serial No.: 09/770,410
Filing Date: January 25, 2001
Page 3

Listing of Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1-50 (canceled).

Claim 51 (original): A device for separating a mixture of polynucleotides, said device comprising:

a polymeric monolith having non-polar chromatographic surfaces,

wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer, wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

Claim 52 (original): A device of claim 51 wherein said tube is devoid of retaining frits.

Attorney Docket No.: P-756 (TI-0022)
Inventors: Huber et al.
Serial No.: 09/770,410
Filing Date: January 25, 2001
Page 4

Claim 53 (original): A device of claim 51 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

Claim 54 (original): A device of claim 53 wherein said theoretical plates per meter is determined from the retention time of single stranded p(dT)₁₈ standard using the following equation:

$$(N/L) = (5.54/L) \left(\frac{t_R}{w_{0.5}} \right)^2$$

wherein N is the number of theoretical plates, t_R is the retention time of said standard determined during an isocratic elution, $w_{0.5}$ is the peak width at half height, and L is the length of the monolith in meters.

Claim 55 (original): A device of claim 54 wherein said tube has an inner diameter of 200 micrometer and a length of 60mm, wherein during said isocratic elution said monolith has a back pressure in the range of 180 to 200 bar, and a flow rate in the range of 2 to 3 $\mu\text{L}/\text{min}$ at an elution temperature of 50°C.

Attorney Docket No.: **P-756 (TI-0022)**
Inventors: **Huber et al.**
Serial No.: **09/770,410**
Filing Date: **January 25, 2001**
Page 5

Claim 56 (original): A device of claim 51 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

Claim 57 (original): A device of claim 51 wherein the chromatographic surfaces of said monolith are devoid of micropores.

Claim 58 (original): A device of claim 57 wherein said monolith has channels sufficiently large for convective flow of said mobile phase.

Claim 59 (original): A device for separating a mixture of polynucleotides, said device comprising:

a polymeric monolith having non-polar chromatographic surfaces,

wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is contained within a fused silica tube, and

Attorney Docket No.: **P-756 (TI-0022)**
Inventors: **Huber et al.**
Serial No.: **09/770,410**
Filing Date: **January 25, 2001**
Page 6

wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

Claim 60 (original): A device of claim 59 wherein said tube has an inner diameter in the range of 1 micrometer to 1000 micrometer.

Claim 61 (original): A device of claim 59 wherein said tube is devoid of retaining frits.

Claim 62 (original): A device of claim 59 wherein said monolith is characterized by having 10,000 to 200,000 theoretical plates per meter.

Claim 63 (currently amended): A device of claim 59 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that ~~resembles the surface said surface morphology of said monolith~~ is brush-like.

Claim 64 (original): A device of claim 59 wherein said monolith comprises an underivatized monolithic stationary phase.

Attorney Docket No.: **P-756 (TI-0022)**
Inventors: **Huber et al.**
Serial No.: **09/770,410**
Filing Date: **January 25, 2001**
Page 7

Claim 65 (original): A device of claim 59 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

Claim 66 (original): A device of claim 59 wherein said monolith is devoid of micropores and wherein said monolith has channels sufficiently large for convective flow of said mobile phase.

Claim 67 (original): A device for separating a mixture of polynucleotides, said device comprising:

a polymeric monolith having non-polar chromatographic surfaces,

wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is contained within a fused silica tube,

wherein said tube has been silanized, and

wherein said tube is devoid of retaining frits.

Attorney Docket No.: **P-756 (TI-0022)**
Inventors: **Huber et al.**
Serial No.: **09/770,410**
Filing Date: **January 25, 2001**
Page 8

Claim 68 (original): A device of claim 67 wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

Claim 69 (original): A device of claim 67 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

Claim 70 (original): A device of claim 67 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like.

Claim 71 (original): A device of claim 67 wherein said tube has an inner diameter in the range of 1 micrometer to 1000 micrometer.

Claim 72 (original): A device of claim 67 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of

Attorney Docket No.: **P-756 (TI-0022)**
Inventors: **Huber et al.**
Serial No.: **09/770,410**
Filing Date: **January 25, 2001**
Page 9

octadecyl modified poly(styrene-divinylbenzene) particles,
wherein said surface morphology of said monolith is rugulose.

Claim 73 (currently amended): A device for separating a
mixture of polynucleotides, said device comprising:

a polymeric monolith having non-polar chromatographic
surfaces,

wherein said monolith comprises an underivatized polystyrene
divinylbenzene matrix,

wherein said monolith is contained within a tube having an
inner diameter in the range of 1 micrometer to 1000 micrometer,

wherein said monolith is characterized by having 10,000 to
200,000 theoretical plates per meter.

Claim 74 (currently amended): A device of claim 73 wherein
said monolith is contained within a tube having an inner diameter
in the range of ~~1~~ 10 micrometer to ~~1000~~ 500 micrometer.

Claim 75 (original): A device of claim 73 wherein said
monolith is immobilized by covalent attachment at the inner wall
of said tube.

Attorney Docket No.: **P-756 (TI-0022)**
Inventors: **Huber et al.**
Serial No.: **09/770,410**
Filing Date: **January 25, 2001**
Page 10

Claim 76 (original): A device of claim 75 wherein said tube is devoid of retaining frits.

Claims 77-78 (canceled).

Claim 79 (original): A device for separating a mixture of polynucleotides, said device comprising:

a polymeric monolith having non-polar chromatographic surfaces,

wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is characterized by having at least 100,000 theoretical plates per meter,

wherein said monolith is contained within a silanized fused silica tube having an inner diameter in the range of 10 micrometer to 1000 micrometer,

wherein said monolith is immobilized at the inner wall of said tube.

Claim 80 (original): A device of claim 79 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

Attorney Docket No.: **P-756 (TI-0022)**
Inventors: **Huber et al.**
Serial No.: **09/770,410**
Filing Date: **January 25, 2001**
Page 11

Claim 81 (original): A device of claim 79 wherein said monolith is contained within a tube having an inner diameter in the range of 1 micrometer to 1000 micrometer.

Claims 82-83 (canceled).

Claim 84 (original): A device of claim 79 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

Claim 85 (original): A miniaturized chromatographic system for separating a mixture of polynucleotides said system comprising the device of claim 79.

Claim 86 (original): A device for separating a mixture of polynucleotides, said device comprising:

a polymeric monolith having non-polar chromatographic surfaces,

wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the

Attorney Docket No.: **P-756 (TI-0022)**
Inventors: **Huber et al.**
Serial No.: **09/770,410**
Filing Date: **January 25, 2001**
Page 12

surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose and brush-like,

wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer,

wherein said monolith is immobilized at the inner wall of said tube.

Claim 87 (original): A device of claim 86 wherein said tube is devoid of retaining frits.

Claim 88 (original): A device of claim 86 wherein said monolith characterized by having 100,000 to 200,000 theoretical plates per meter.

Claim 89 (original): A device of claim 86 wherein said tube has been silanized.

Attorney Docket No.: **P-756 (TI-0022)**
Inventors: **Huber et al.**
Serial No.: **09/770,410**
Filing Date: **January 25, 2001**
Page 13

Claim 90 (original): A device of claim 86 wherein said surfaces of said monolith are non-porous.

Claim 91 (original): A device of claim 86 wherein said monolith is formed from a polymerization mixture including underivatized styrene, a crosslinking agent, and a porogen, wherein said porogen comprises tetrahydrofuran.

Claim 92 (original): A device of claim 86 wherein said polynucleotides comprise double-stranded fragments having lengths in the range of 3 to 600 base pairs.

Claims 93-94 (canceled).

Claim 95 (original): A system of claim 85 wherein said monolith is operatively coupled to a mass spectrometer.

Claim 96 (canceled.)

Claim 97 (original): A chromatographic device, said device comprising:

Attorney Docket No.: **P-756 (TI-0022)**
Inventors: **Huber et al.**
Serial No.: **09/770,410**
Filing Date: **January 25, 2001**
Page 14

a polymeric monolith having non-polar chromatographic surfaces,

wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is contained within a silanized fused silica tube having an inner diameter in the range of 10 micrometer to 1000 micrometer, and wherein said monolith is immobilized at the inner wall of said tube.